

MRID No. 422563-03

DATA EVALUATION RECORD

1. **CHEMICAL:** NTN 33893.
Shaughnessey No. 129059.
2. **TEST MATERIAL:** NTN 33893 technical; ABC Reference No. TS-4204; a light yellow powder.
3. **STUDY TYPE:** 72-2. Freshwater Invertebrate Static Acute Toxicity Test. Species Tested: *Hyalella azteca*.
4. **CITATION:** England, D. and J.D. Bucksath. 1991. Acute Toxicity of NTN 33893 to *Hyalella azteca*. Report No. 101960. Prepared by ABC Laboratories, Inc., Columbia, MO. Submitted by Mobay Corporation, Stilwell, KS. EPA MRID No. 422563-03.
5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Louis M. Rifici*
Date: *9/28/92*
Dan Fentress
11/25/92 EFED/EEB
6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *P. Kosalwat*
Date: *9/28/92*
Pim Kosalwat
10/14/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature:
Date:
7. **CONCLUSIONS:** The study is scientifically sound but does not meet the guideline requirements for a static acute toxicity test using freshwater invertebrates. *Hyalella azteca* is not a recommended species in the SEP. The authors do not provide any justification for its use. In addition, the purity of the test material was not reported. The 48- and 96-hour EC_{50} values were 115.3 $\mu\text{g/l}$ and 55 $\mu\text{g/l}$ (mean measured concentrations), respectively. Therefore NTN 33893 is classified as highly toxic or very highly toxic to *H. azteca* depending on which LC_{50} is used. The 48- and 96-hour NOEC values were 0.97 $\mu\text{g/l}$ and 0.35 $\mu\text{g/l}$ mean measured concentrations. *(96-hr LC_{50} is used with this test species, GS)*
8. **RECOMMENDATIONS:** The registrant should provide justification for using *H. azteca*, the registrant must also

provide the lot/batch number and percentage active ingredient for the test material and the age of the test organisms used. Justification is not necessary for using H. azteca, the information will be used as supplemental data.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile *Hyalella azteca* (2-3 mm long) used in the test were obtained from in-house cultures. Adults were acclimated to the hard blended test water over a period of several days. The culture vessels were 1-gallon glass jars containing hard maple leaves as a primary food/substrate. A supplement of fish food, cereal leaves, and yeast was added 2-3 times weekly. The temperature was 20°C and the photoperiod was 16 hours of light.

B. **Test System:** Vessels used in the test were glass beakers containing 1000 ml of test solution. A 2" by 6" piece of nylon screen was placed in each test vessel as a substrate for the test animals. The beakers were placed in a water bath maintained at 20 ±2.0°C. Lighting was the same as that used in culturing.

Blended hard water (a well water and reverse-osmosis water mixture) with a hardness of 180 mg/l as CaCO₃, an alkalinity of 194 mg/l as CaCO₃, a pH of 8.3, and a conductivity of 430 µmhos/cm, was used as dilution water.

Two stock solutions (0.0001 mg/ml and 0.10 mg/ml) were prepared.

C. **Dosage:** Ninety-six-hour, static test. Based on preliminary testing, nine nominal concentrations (0.33, 1.0, 3.3, 10, 33, 100, 330, 1000, and 3000 µg/l) and a dilution water control were used.

D. **Design:** Ten *H. azteca* were impartially distributed to each test beaker. Two beakers were used per test level. The loading was approximately one organism per 100 ml of solution. All beakers were observed once daily to determine survival and abnormal effects.

The temperature, dissolved oxygen concentration (DO), and pH were measured in one replicate of the control, low, two middle, and high test concentrations daily.

The temperature of the water bath was continuously monitored using a data logger.

Measured concentrations of NTN 33893 in the test solutions were determined at test initiation and termination using high performance liquid chromatography.

- E. **Statistics:** The LC_{50} values and associated confidence intervals were determined using a computer program developed by Stephan et al. (1977).

12. **REPORTED RESULTS:** The mean measured concentrations were 0.35, 0.97, 3.5, 10, 34, 100, 340, 1000, and 3100 $\mu\text{g/l}$ and averaged 102% of nominal concentrations (Table 5, attached). "The test material appeared to be stable in the system based on information supplied by the study sponsor and the consistent measurements at 0 and 96 hours."

The 48-hour LC_{50} value could not be determined due to insufficient mortality (Table 6, attached). The 96-hour LC_{50} was 526 $\mu\text{g/l}$ (95% C.I. = 194-1263 $\mu\text{g/l}$) using the moving average method. The 48 and 96-hour EC_{50} values were 129 (95% C.I. = 85-193 $\mu\text{g/l}$) and 55 $\mu\text{g/l}$ (95% C.I. = 34-93 $\mu\text{g/l}$), respectively (Table 7, attached). The 96-hour no-observed-effect concentration (NOEC) was 0.35 $\mu\text{g/l}$, based on the lack of mortality and abnormal effects at this level (Table 3, attached).

During the test, the temperature remained constant at 20°C. Dissolved oxygen concentrations ranged from 5.2 to 8.2 mg/l (60 to 94% of saturation at 20°C). The pH was 8.0-8.4.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
The authors did not present any conclusions.

Quality assurance and study compliance statements were included in the report, indicating that the study was conducted in accordance with USEPA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

- A. **Test Procedure:** The test procedures were in accordance with the SEP, except for the following:

Hyalella azteca is not a recommended species. The authors present no justification for using this species. In addition, the age and developmental stage of the organisms were not reported. It is possible

that 2 or more instars were present in the test population.

The test material was not adequately described. No lot or batch number or percentage active ingredient was provided in the report.

The recommended test temperature for amphipods is 17°C. The temperature during this test was 20°C.

The procedures used to prepare the test solutions and the time between test solution preparation and test initiation were not reported.

Fifteen to 30-minute dawn and dusk simulation periods are recommended in the SEP. These simulations were not used during the test.

The test concentrations were approximately 30% of the next highest concentration. The SEP recommends that each nominal concentration be at least 60% of next highest.

The dimensions of the test vessels were not reported.

- B. Statistical Analysis: The reviewer calculated the 48 and 96-hour EC_{50} values using EPA's Toxanal computer program. The results were similar to those of the authors' (see attached printouts 1 and 2).
- C. Discussion/Results: The study is scientifically sound but does not meet the guideline requirements for a static acute toxicity test using freshwater invertebrates. *Hyalella azteca* is not a recommended species in the SEP. The authors do not provide any justification for its use. In addition, the purity of the test material was not reported. The 48- and 96-hour EC_{50} values were 115.3 $\mu\text{g/l}$ and 55 $\mu\text{g/l}$ (mean measured concentrations), respectively. Therefore NTN 33893 is classified as highly toxic or very highly toxic to *H. azteca* depending on which LC_{50} is used. The 48- and 96-hour NOEC values were 0.97 $\mu\text{g/l}$ and 0.35 $\mu\text{g/l}$ mean measured concentrations. (96-hr values used with this test species) as
- D. Adequacy of the Study:
- (1) Classification: Supplemental.
 - (2) Rationale: The test species used is not recommended in the SEP. The authors do not

provide any justification for its use. In addition, the percent active ingredient of the test material was not reported.

(3) Repairability: No.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 09-16-92.

TABLE 3

Individual Mortality and Behavioral Observations for the Static Acute
Toxicity Exposure of *Hyaella azteca* to NTN-33893

Mean Measured Concentration ($\mu\text{g/L}$)	# Test Org.	24-Hour		48-Hour		72-Hour		96-Hour	
		Cum. Mort.	Obs.	Cum. Mort.	Obs.	Cum. Mort.	Obs.	Cum. Mort.	Obs.
Control	20	0	20 N	0	20 N	0	20 N	0	20 N
0.35	20	0	20 N	0	20 N	0	20 N	0	20 N
0.97	20	0	20 N	0	20 N	0	20 N	1	19 N
3.5	20	0	20 N	0	5 Le; 14 N; 1 Sur	0	4 Le; 13 N; 3 Sur	1	8 Le; 9 N; 2 Sur
10	20	0	3 Le; 1 Sur; 16 N	1	11 Le; 8 N	1	17 Le; 2 Sur	1	19 Le
34	20	1	1 Im; 12 Le; 4 Sur; 2 N	1	1 Im; 14 Le; 4 Sur	1	1 Im; 16 Le; 2 Sur	1	5 Im; 14 Le
100	20	0	4 Im; 11 Le; 5 Sur	0	10 Im; 9 Le; 1 Sur	0	8 Im; 12 Le	2	6 Im; 12 Le
340	20	1	6 Im; 8 Le; 5 Sur	1	12 Im; 6 Le; 1 Sur	4	13 Im; 3 Le	8	11 Im; 1 Le
1100 1000	20	0	20 Im	6	14 Im	11	9 Im	16	4 Im
3100	20	1	19 Im	3	17 Im	11	9 Im	12	8 Im

N = Normal; Im = Immobile; Sur = Surfacing; Le = Lethargic.

TABLE 5

Measured Concentrations of NTN-33893 During the Static Acute Toxicity Test with *Hyallela azteca*

Sample	Nominal Concentration ($\mu\text{g/L}$)	Measured Concentration ¹ (mg/L)		Mean ²	% Recovery ²
		0-Hour	96-Hour		
Reagent Blank	---	<0.72	<0.72	<0.72	---
Control	---	<0.18	<0.18	<0.18	---
Level 1	0.33	0.33	0.36	0.35	106
Level 2	1.0	0.97	0.97	0.97	97
Level 3	3.3	3.6	3.3	3.5	106
Level 4	10	11	9.4	10	102
Level 5	33	36	32	34	103
Level 6	100	110	94	100	101
Level 7	330	350	320	340	101
Level 8	1000	1100	970	1000	103
Level 9	3000	3200	2900	3100	102
Stock 1	0.10 mg/mL	0.12 mg/mL	---	---	120
Stock 2	0.0001 mg/mL	0.0001 mg/mL	---	---	100

Mean \pm S.D. = 102 \pm 3 %

Values rounded to two significant digits for reporting.

Mean and % recovery calculated from measured values, means rounded for reporting.

TABLE 6

The Acute Toxicity of NTN-33893 to *Hyalella azteca*^a Based on Mortality

Mean Measured Conc. (mg/L)	Percent Mortality			
	24 Hours	48 Hours	72 Hours	96 Hours
Control	0	0	0	0
0.35	0	0	0	0
0.97	0	0	0	5
3.5	0	0	0	5
10	0	5	5	5
34	5	5	5	5
100	0	0	0	10
340	5	5	20	40
1000	0	30	55	80
3100	5	15	55	60
LC ₅₀ (μg/L)	--- ^b	--- ^b	1756	526
95 % Confidence Limits (μg/L)	---	---	884-5448	194-1263
LC ₅₀ calculated using:	---	---	Probit Method	Moving Average

Bioassay conducted at 20°C.

LC₅₀ values and their 95 % confidence limits could not be calculated due to insufficient mortality.

The 96-hour no-effect concentration was 0.35 μg/L, based on the lack of mortality at that test level.

TABLE 7

The Acute Toxicity of NTN-33893 to *Hyalella azteca*^a Based on Immobilization

Mean Measured Conc. (mg/L)	Percent Immobilization			
	24 Hours	48 Hours	72 Hours	96 Hours
Control	0	0	0	0
0.35	0	0	0	0
0.97	0	0	0	5
3.5	0	0	0	5
10	0	5	5	5
34	10	10	10	30
100	20	50	40	40
340	35	65	85	95
1000	100	100	100	100
3100	100	100	100	100
EC ₅₀ (μg/L)	218	129	113	55
95% Confidence Limits (μg/L)	148-324	85-193	77-165	34-93
EC ₅₀ calculated using:	Moving Average	Probit Method	Probit Method	Moving Average

Bioassay conducted at 20°C.

The 96-hour no-effect concentration was 0.35 μg/L, based on the lack of mortality and abnormal effects at that test level.

48-hour EC50

Printout 1

RIFICI NTN 33893 HYALELLA AZTECA 09-16-92

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3100	20	20	100	9.536742E-05
1000	20	20	100	9.536742E-05
340	20	13	65	13.1588
100	20	10	50	58.80985
34	20	2	10	2.012253E-02
10	20	1	5	2.002716E-03
3.5	20	0	0	9.536742E-05
.97	20	0	0	9.536742E-05
.35	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 34 AND 1000 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 99.99999

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
6	.0513501	115.2767	74.90048 179.5673

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.0796061	1	.6815431

SLOPE = 1.821192
95 PERCENT CONFIDENCE LIMITS = 1.307351 AND 2.335033

LC50 = 127.7236
95 PERCENT CONFIDENCE LIMITS = 84.80026 AND 192.385

LC10 = 25.64045
95 PERCENT CONFIDENCE LIMITS = 11.76405 AND 42.33426

96-hou EC50

Printout 2

RIFICI NTN 33893 HYALELLA AZTECA 09-16-92

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3100	20	20	100	9.536742E-05
1000	20	20	100	9.536742E-05
340	20	19	95	2.002716E-03
100	20	8	40	25.17223
34	20	6	30	5.765915
10	20	1	5	2.002716E-03
3.5	20	1	5	2.002716E-03
.97	20	1	5	2.002716E-03
.35	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 10 AND 340 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 121.054

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
8	5.135013E-02	← 56.56807	34.35538 - 98.85121

98.85121

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.2847988	2.965891	4.14002E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.479745
95 PERCENT CONFIDENCE LIMITS = .690056 AND 2.269433

LC50 = 67.48288
95 PERCENT CONFIDENCE LIMITS = 23.19101 AND 198.031

LC10 = 9.352712
95 PERCENT CONFIDENCE LIMITS = .6949445 AND 26.30639
